WEST

Generate Collection | Print

L3: Entry 36 of 65 File: USPT May 29, 2001

DOCUMENT-IDENTIFIER: US 6238893 B1
TITLE: Method for intracellular DNA amplification

Brief Summary Text (15):
As stated before, all adenovirus vectors currently used in gene therapy are believed to have a deletion in the E1 region, where novel genetic information can be introduced. The E1 deletion renders the recombinant virus replication defective (Stratford-Perricaudet and Perricaudet, 1991). We have demonstrated that recombinant adenoviruses are able to efficiently transfer recombinant genes to the rat liver and airway epithelium of rhesus monkeys (Bout et al., 1994b; Bout et al., 1994a). In addition, we (Vincent et al., 1996a; Vincent et al., 1996b) and others (see, e.g, Haddada et al., 1993) have observed a very efficient in vivo adenovirus mediated gene transfer to a variety of tumor cells in vitro and to solid tumors in animals models (lung tumors, glioma) and human xenografts in immunodeficient mice (lung) in vivo (reviewed by Blaese et al., 1995).

Brief Summary Text (44):
6) New adenovirus vectors with extended E1 deletions (deletion nt. 459-3510). Those viral vectors lack sequences homologous to E1 sequences in said packaging cell lines. These adenoviral vectors contain pIX promoter sequences and the pIX gene, as pIX (from its natural promoter sequences) can only be expressed from the vector and not by packaging cells (Matsui et al., 1986, Hoeben and Fallaux, pers.comm.; Imler et al., 1996).

Detailed Description Text (27):
The newly developed adenovirus vectors harboring an E1 deletion of nt. 459-3510 will be used for gene transfer purposes. These vectors are also the basis for the development of further deleted adenovirus vectors that are mutated for, for example, E2A, E2B or E4. Such vectors will be generated, for example, on the newly developed packaging cell lines described above.

Detailed Description Text (46):
We have generated a cell line that harbors E1 sequences of adenovirus type 5 ("Ad5"), able to trans-complement E1 deleted recombinant adenovirus (Fallaux et al., 1996). This cell line was obtained by transfection of human diploid human embryonic retinoblasts ("HER") with pAd5XhoIC, that contains nt. 80-5788 of Ad5; one of the resulting transformants was designated 911. This cell line has been shown to be very useful in the propagation of E1 defective recombinant adenovirus. It was found to be superior to 293 cells. Unlike 293 cells, 911 cells lack a fully transformed phenotype, which most likely is the cause of its better performance as adenovirus packaging line:

Detailed Description Text (52):
Adenovirus sequences are derived either from pAd5.SalB, containing nt. 80-9460 of human adenovirus type 5 (Bernards et al., 1983) or from wild-type Ad5 DNA. pAd5.SalB was digested with SalI and XhoI and the large fragment was religated and this new clone was named pAd5.X/S. The pTN construct (constructed bv Dr. R. Vogels, IntroGene, Leiden, The Netherlands) was used as a source for the human PGK promoter and the NEO gene.

Detailed Description Text (130):
Plasmid pICLI was created from plasmid pICL by insertion of the SalI-SgrAI fragment from pICL, containing the Ad5-ITR into the Asp718 site of pICL. The 194 bp

SalI-SgrAI fragment was isolated from pICL, and the cohesive ends were converted to blunt ends using E. coli DNA polymerase I (Klenow fragment) and dNTP's. The Asp718 cohesive ends were converted to blunt ends by treatment with mungbean nuclease. By ligation clones were generated that contain the ITR in the Asp718 site of plasmid pICL. A clone that contained the ITR fragment in the correct orientation was designated pICLI (FIG. 18). Generation of adenovirus Ad-CMV-hcTK. Recombinant adenovirus was constructed according to the method described in EPO Patent application 95202213. Two components are required to generate a recombinant adenovirus. First an adaptor-plasmid containing the left terminus of the adenovirus genome containing the ITR and the packaging signal, an expression cassette with the gene of interest, and a portion of the adenovirus genome which can be used for homologous recombination. In addition, adenovirus DNA is needed for recombination with the aforementioned adaptor plasmid. In the case of Ad-CMV-hcTK, the plasmid PCMV.TK was used as a basis. This plasmid contains nt. 1-455 of the adenovirus type 5 genome, nt. 456-1204 derived from pCMV.beta. (Clontech, the PstI-StuI fragment that contains the CMV enhancer promoter and the 16S/19S intron from Simian Virus 40), the HSV TK gene (described in EPO Patent application 95202213), the SV40-derived polyadenylation signal (nt 2533-2668 of the SV40 sequence), followed by the BglII-ScaI fragment of Ad5 (nt. 3328-6092 of the Ad5 sequence). These fragments are present in a pMLP10-derived (Levrero et al., 1991) backbone. To generate plasmid pAD-CMVhc-TK, plasmid pCMV.TK was digested with ClaI (the unique ClaI-site is located just upstream of the TK open readingframe) and dephosphorylated with Calf-Intestine Alkaline Phosphate. To generate a hairpin-structure, the synthetic oligonucleotides HP/cla1 (SEQ ID NO: 19) and HP/cla2 (SEQ ID NO: 20) were annealed and phosphorylated on their 5-OH groups with T4-polynucleotide kinase and ATP. The double-stranded oligonucleotide was ligated with the linearized vector fragment and used to transform E. coli strain "Sure". In section of the oligonucleotide into the ClaI site will disrupt the ClaI recognition sites. The oligonucleotide contains a new ClaI site near one of its termini. In selected clones, the orientation and the integrity of the inserted oligonucleotide was verified by sequence analyses. A clone containing the oligonucleotide in the correct orientation (the ClaI site at the ITR side) was denoted pAd-CMV-hcTK. This plasmid was co-transfected with ClaI digested wild-type Adenovirus-type5 DNA into 911 cells. A recombinant adenovirus in which the CMV-hcTK expression cassette replaces the E1 sequences was isolated and propagated using standard procedures.

Detailed Description Text (140):
The following demonstrates that DNA molecules which contain nucleotides 3510-35953 (viz. 9.7-100 map units) of the adenovirus type 5 genome (thus lack the E1 protein-coding regions, the right-hand ITR and the encapsidation sequences) and a terminal DNA sequence that is complementary to a portion of the same strand of the DNA molecule when present in single-stranded form other than the ITR, and as a result is capable of forming a hairpin structure, can replicate in 911 cells.

Detailed Description Text (144):
The following demonstrates that DNA molecules which contain nucleotides 3503-35953 (viz. 9.7-100 map units) of the adenovirus type 5 genome (thus lack the E1 protein-coding regions, the right-hand ITR and the encapsidation sequences) and a terminal DNA sequence that is complementary to a portion the same strand of the DNA molecule other than the ITR, and as a result is capable of forming a hairpin structure, can replicate in 911 cells and can provide the helper functions required to encapsidate the pICLI and pICLhac derived DNA fragments.

Detailed Description Text (151):
Bernards, R., Schrier, P. I., Bos, J. L., and Eb, A. J. v. d. (1983): Role of adenovirus types 5 and 12 early region 1b tumor antigens in oncogenic transformation. Virology 127, 45-53.

Detailed Description Text (152):
Bett, A. J, Prevec, L., and Graham, F. L. (1993): Packaging Capacity and Stability of Human Adenovirus Type-5 Vectors. J Virol 67, 5911-5921.

Detailed Description Text (168):
Grable, M., and Hearing, P. (1990): Adenovirus type 5 packaging domain is composed of a repeated element that is functionally redundant. J. Virol. 64, 2047-2056.

Detailed Description Text (169):
Grable, M., and Hearing, P. (1992): cis and trans Requirements for the Selective Packaging of Adenovirus Type-5 DNA. J. Virol 66, 723-731.

Detailed Description Text (171):
Graham, F. L., Smiley, J., Russell, W. C., and Naira, R. (1977): Characteristics of a human cell line transformed by DNA from adenovirus type 5. J. Gen. Virol. 36, 59-72.

Detailed Description Text (174):
Hearing, P., Samulski, R. J., Wishart, W. L., and Shenk, T. (1987): Identification of a repeated sequence element required for efficient encapsidation of the adenovirus type 5 chromosome. J. Virol. 61, 2555-2558.

Detailed Description Text (181):
Kruijer, W., Nicolas, J. C., Schaik. F. M. v., and Sussenbach, J. S. (1983): Structure and function of DNA binding proteins from revertants of adenovirus type 5 mutants with a temperature-sensitive DNA replication. Virology 124, 425-433.

Detailed Description Text (193):
Rice, S. A., and Klessig, D. F. (1985): Isolation and analysis of adenovirus type 5 mutants containing deletions in the gene encoding the DNA-binding protein. J. Virol. 56, 767-778.

Other Reference Publication (9):
#Carovokyri et al., "Constitutive Episomal Expression of Polypeptide IX (pIX) in a 293-Based Cell Line Complements the Deficiency of pIX Mutant Adenovirus Type 5", Journal of Virology, 69(11):6627-6633, Nov. 1995.

Other Reference Publication (20):
#Krougliak et al., "Development of Cell Lines Capable of Complementing E1, E4, and Protein IX Defective Adenovirus Type 5 Mutants", Human Gene Therapy, 6:1575-1586, Dec. 1995.

Other Reference Publication (24):
#Schaack et al., "Adenovirus Type 5 Precursor Terminal Protein-Expressing 293 and HeLa Cell Lines", Journal of Virology, 69(7):4079-4085, Jul. 1995.

Other Reference Publication (30):
Bernards, Rene, et al., Role of Adenovirus Types 5 and 12 Early Region 1b Tumor Antigens in Oncogenic Trahsformation, Virology 127, pp. 45-53 (1983).

Other Reference Publication (31):
Graham, F.L., et al., Characteristics of a Human Cell Line Transformed by DNA from Human Adenovirus Type 5, J. gen. Virol., 36, pp. 59-74, (1977).

Other Reference Publication (34):
Louis, Nathalie, et al., Cloning and Sequencing of the Cellular-Viral Junctions from the Human Adenovirus Type 5 Transformed 293 Cell Line, Virology 233, pp. 423-429 (1997).

Other Reference Publication (35):
Roberts, Bryan E., et al., Individual Adenovirus Type 5 Early Region 1A Gene Products Elicit Distinct Alterations of Cellular Morphology and Gene Expression, Journal of Virology, pp. 404-413, Nov. 1985.

Other Reference Publication (45):
Vos et al., "Characterization of Adenovirus Type 5 Insertion and Deletion Mutants Encoding Altered DNA Binding Proteins", Virology, 172, pp. 634-642, 1989.

WEST

Generate Collection | Print

L3: Entry 42 of 65 File: USPT Oct 31, 2000

DOCUMENT-IDENTIFIER: US 6140087 A
TITLE: Adenovirus vectors for gene therapy

Brief Summary Text (9):
The representative Adenovirus 5 ("Ad5") genome used in embodiments of the present invention is a 36 kb linear duplex. Its sequence has been published. (Chroboczek, J., Bieber, F., and Jacrot, B. (1992) The Sequence of the Genome of Adenovirus Type 5 and Its Comparison with the Genome of Adenovirus Type 2, Virology 186, 280-285; hereby incorporated by reference). The Ad5 genome contains a 100-150 base pair (bp) inverted terminal repeat ("ITR") at each end of the linearized genome. A terminal protein ("TP") of 55,000 daltons is covalently linked to the 5' end of each strand. Both the TP and the ITRs are thought to play a role in viral DNA replication. McGrory, W. J. et al. (1988), Virology, 163, 614-617 and Ghosh-Choudhury, G. et al. (1986), Gene, 50, 161-171 (hereby incorporated by reference). Ad5 has infected each human cell line tested, although some cells, such as lymphocytes, are relatively nonpermissive.

Brief Summary Text (11):
The Ad virion has the ability to package up to 105-106% of the wild type genome length. Bett, A. J., Prevec, L., & Graham, F. L. (1993) Packaging Capacity and Stability of Human Adenovirus Type 5 Vectors, J. Virol. 67: 5911-5921. Larger genomes (e.g., 108% of the wild type in size), result in instability of the virus and poor growth rates. Id. This packaging ability allows the insertion of only approximately 1.8-2.0 kb of excess DNA into the Ad genome.

Brief Summary Text (12):
To package larger inserts, it is necessary to first delete portions of the viral genome. Parts of region E1 can be deleted, and the resulting viruses can be propagated in human 293 cells. (293 cells contain and express E1, complementing viral mutants that are defective in E1.) Foreign nucleic acids can be inserted in place of E1, in Ad5 genomes that contain E1 deletions of up to 2.9 kb, to yield conditional helper-independent vectors with a capacity for inserts of 4.7-4.9 kb.

Brief Summary Text (17):
One embodiment of the present invention provides a bacterial plasmid comprising a circularized modified human adenovirus type 5 (Ad5) genome. The nucleotide sequence of the plasmid has a deletion within early region 3 (E3) of said Ad5 genome, and a segment of bacterially replicable pBR322 plasmid encoding ampicillin resistance substituted for a sequence of early region 1A (E1A) that corresponds, in whole or in part, to the packaging signal.

Brief Summary Text (18):
Another embodiment provides a bacterial plasmid comprising approximately 340 base pairs from the left end of the adenovirus type 5 genome, the left end inverted terminal repeat sequences of said genome and the packaging signal sequences thereof, said plasmid comprising also a eukaryotic gene sequence of up to about 8 kilobases foreign to said plasmid and to said viral genome. The adenovirus sequence from approximately nucleotide position 3540 thereof to approximately position 5790 thereof is present on the right side of said foreign sequence.

Brief Summary Text (19):
Other embodiments of the present invention include adenovirus genome constructs containing E1 deletions and foreign inserts of eukaryotic origin, using any

combination of size of E1 deletion and/or of size of foreign insert that can be accommodated in the plasmid and still remain operable. Because of the large capacity of the vectors provided herein, multiple inserts of foreign genes can be placed in the E1 cloning site. For example, two or more genes encoding different antigens, or genes encoding useful proteins, can be combined with genes encoding chemically selectable markers.

Drawing Description Text (7):
FIG. 4 is a diagrammatic representation of the structure and construction of a 3.2 kb E1 deletion, and two examples (p.DELTA.E1sp1A and p.DELTA.E1sp1B) of plasmids that contain said deletion.

Drawing Description Text (8):
FIG. 5 illustrates the different levels of protein IX synthesized using plasmids having different E1 deletions with or without a reintroduced Ssp1 site.

Drawing Description Text (9):
FIG. 6 illustrates heat stability of viruses with the 3.2 kb E1 deletion with or without a reintroduced Ssp1 site.

Drawing Description Text (11):
FIG. 8 depicts the strategy for the construction of a double recombinant containing lacZ in the E3 deletion and firefly luciferase in the E1 deletion.

Detailed Description Text (16):
While a wide variety of host cells are contemplated, certain embodiments require that the host cell express E1 sequences that are missing from or inactivated in the vector. While the human 293 cell line is the preferred host cell, the invention also contemplates other cell lines capable of complementing the vector having an E1 deletion. It is important to recognize that the present invention is not limited to the use of such cells as are used herein. Cells from different species (human, mouse, etc.) or different tissues (breast epithelium, colon, neuronal tissue, lymphocytes, etc.) may also be used.

Detailed Description Text (32):
Cell culture media and reagents were obtained from GIBCO Laboratories (Grand Island, N.Y.). Adenovirus (Ad) vectors were tittered and passaged on 293 cells which constitutively express the left 11% of the Ad5 genome, comprising the E1 region. Graham, F. L., J. Smiley, W. C. Russell, and R. Nairn. 1977. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J. Gen. Virol. 36:59-72. The 293 cells were grown :in monolayer in F-11 minimum essential medium supplemented with 100 units penicillin/ml, 100 .mu.g streptomycin/ml, 2.5 .mu.g amphotericin/ml and with 10% newborn calf serum for cell maintenance or 5% horse serum for virus infection. KB cells grown in spinner culture were maintained in Joklik's modified medium supplemented with antibiotics as above and with 10% horse serum.

Detailed Description Text (57):
Plasmids pBHG10, pBHG11 and pBHGE3 were designed so that they would contain all the essential Ad5 sequences required to produce infectious virus upon transfection of 293 cells except for the packaging signal (194-358 bp) needed to encapsidate viral DNA into viral particles. To generate infectious viral vectors pBHG10, pBHG11, pBHGE3 or derivatives carrying an insert in E3 must be cotransfected into 293 cells with a second plasmid containing left end (E1) viral sequences including the packaging signal, as illustrated in FIG. 3. To maximize the capacity of the BHG vector system we required a plasmid with the largest possible E1 deletion for co-transfections with the BHG plasmids.

Detailed Description Text (58):
Our analysis of E1 sequences revealed that a deletion of approximately 3.2 kb could be created by removing the sequences between an Ssp I site at 339 bp and an Afl II site at 3533 bp (FIG. 4). This deletion does not interfere with the ITR (1-103 bp), the essential core packaging signal (194-358 bp) or coding sequences for protein IX but it does remove the sp1 binding site (3525-3530 bp) from the protein IX promoter. While this 3.2 kb E1 deletion does not interfere with the E1 enhancer region, it

does remove the 3'-most, packaging element. The removal of this element has little or no effect on packaging.

Detailed Description Text (60):
To assess the effect of the 3.2 kb E1 deletion and the reintroduction of the sp1 binding site, we examined protein IX expression by immunoprecipitation. 293 cells were infected at 10 PFU/cell with viruses containing either no deletion in E1 (wild type Ad5), a 2.3 kb deletion extending into the protein IX gene (dl313), the 3.2 kb deletion described above (dl70-3), the 3.2 kb deletion containing the HCMV (AdHCMV2) or .beta.-Actin (Ad.beta.Act2) promoters in the E1 antiparallel orientation or the 3.2 kb deletion containing the HCMV (AdHCMVsp1) or .beta.-Actin (Ad.beta.Actsp1) promoters with the reintroduced sp1 binding site. After labelling with [.sup.35 S]-methionine cell extracts were harvested, samples were immunoprecipitated with anti Ad2 protein IX antibodies and run on a 12% SDS PAGE gel. The results (FIG. 5) indicate that variable levels of protein IX were expressed depending on the sequences upstream from the protein IX gene but with the sp1 site present there was at most a 25% reduction compared to wild type Ad5.

Detailed Description Text (61):
Because protein IX is known to affect the heat stability of virus particles we examined the heat stability of wild type Ad5 compared to dl313, dl70-3, AdHCMV2, Ad.beta.Act2, AdHCMVsp1 and Ad.beta.Actsp1. Stocks of these viruses were titered prior to and after incubation at 45.degree. C. for 1 and 2 hours. Of the six viral mutants tested only dl313 differed significantly in heat lability from wild type (FIG. 6). Even Ad.beta.Act2, which produces only 16% of wild type levels of protein IX (FIG. 5) was as resistant to heat inactivation as was wild type virus. This indicates that Protein IX is likely made in excess during viral infection. We have also found that viruses containing the 3.2 kb E1 deletion replicate in 293 cells to the same final titers as wild type Ad5 (data not shown).

Detailed Description Text (62):
With the verification that the growth characteristics and stability of viruses with the 3.2 kb E1 deletion were not affected it was decided to incorporate this deletion into plasmids p.DELTA.E1sp1A and p.DELTA.E1sp1B for use in cotransfections with the BHG plasmids (FIG. 4). These plasmids contain various restriction sites to facilitate the insertion of foreign genes.

Detailed Description Text (66):
The use of pBHGE3, pBHG10 or pBHG11 combined with the 3.2 kb deletion in E1 should permit rescue of inserts of approximately 5.2, 7.9 and 8.3 kb respectively into viral vectors. In order to test the capacity of the BHG system we constructed an insert of 7.8 kb consisting of the lacZ gene driven by the human cytomegalovirus immediate early promoter and the herpes simplex virus type 1 (HSV-1) gB gene driven by the SV40 promoter in the 3.2 kb E1 deletion (FIG. 7). Following cotransfection of 20-60 mm dishes of 293 cells, 10 with 5 .mu.g each of pBHG10 and pHlacZgBR and the other half with 10 .mu.g of each, one plaque was obtained. This was isolated, expanded, analyzed by restriction digest with HindIII and found to have the expected restriction pattern. The isolate designated AdHlacZgBR was found to express both lacZ and HSV-1 gB at levels comparable to that obtained with vectors containing single inserts of these genes (data not shown).

Detailed Description Text (69):
A shuttle vector, pABS.4, was used in the construction of a double recombinant containing lacZ in the E3 deletion and firefly luciferase in the E1 deletion. The construction of this vector further illustrates the use of the shuttle vectors as well as double recombinants.

Other Reference Publication (14):
N. Jones and T. Shenk, "Isolation of Adenovirus Type 5 Host Range Deletion Mutants Defective for Transformation of Rat Embryo Cells", Cell, 17, 1979, pp. 683-689.

Other Reference Publication (15):
D.S. Bautista et al., "Isolation and Characterization of Insertion Mutants in E1A of Adenovirus Type 5", Virology, 182, 1991, pp. 578-596.

Other Reference Publication (18):
P. Hearing et al., "Identification of a Repeated Sequence Element Required to Efficient Encapsidation of the Adenovirus Type 5 Chromosome", Journal of Virology, 61(8), 1987, pp. 2555-2558.

CLAIMS:

1. A kit for making an adenovirus vector comprising nucleic acid sequences of a first plasmid and a second plasmid, said kit comprising a first and a second plasmid:

(a) wherein said first plasmid comprises:

(i) a modified adenovirus genome, wherein said genome comprises a modification within early region 1 (E1) that comprises a deletion of a packaging signal of the early region 1 which renders said first plasmid incapable of forming viable viral particles in host cells, by eliminating susceptibility of adenoviral sequences encoded by said first plasmid to being encapsidated into a viral particle, but does not affect the ability of the adenovirus genome to replicate, and

(ii) at least one nucleic acid sequence for (A) encoding antibiotic resistance and (B) replication of said first plasmid in host cells; and

(b) wherein said second plasmid is an E1 shuttle plasmid, which comprises at least one nucleic acid sequence comprising adenovirus genome E1 region sufficient to rescue said first plasmid via recombination of said first plasmid and said second plasmid to produce said adenovirus vector.

7. The kit according to claim 1, wherein the E1 deletion in said second plasmid is complemented by viral E1 sequences expressed by 293 cells when said vector is packaged in 293 said cells.

10. The kit according to claim 1 wherein said second plasmid comprises the approximately 340 left end base pairs of the adenovirus type 5 genome, said second plasmid further comprising the left end inverted terminal repeat sequences of said genome and the packaging signal sequences thereof, said second plasmid also comprising a gene sequence of up to about 8 kilobases foreign to said adenovirus vector and to said viral genome, and wherein additional nucleotide sequences sufficient for recombination between said first plasmid and said second plasmid are present on the right side of said foreign sequence.